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Establishment of a complete series of a monosomic tomato chromosome addition lines in the cultivated potato using RFLP and GISH analyses

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Abstract With the aim of establishing a complete monosomic alien tomato chromosome addition series in a potato background, the backcross progenies derived from repeated crossing of potato (+) tomato fusion hybrids to potato were screened through RFLP and GISH analyses. Because of the availability from our previous work of seven of the possible 12 tomato monosomic additions, selected from BC_2 populations, attention was paid to those alien additions that were missing. Thus, since the alien additions were already available for tomato chromosomes 1, 2, 4, 6, 8, 10 and 12, efforts were made to select for chromosomes 3, 5, 7, 9 and 11 by screening specific BC_3 populations. In all, 105 plants from four BC_3 populations were screened through a combination of RFLP and GISH analyses in order to complete the series. Among the newly selected alien addition lines, five were monosomic additions for all the remaining chromosomes and one was a disomic addition for chromosome 11. When using conventional cytogenetics the selection of monosomic alien additions is highly laborious. All the tomato chromosomes showed a variable rate of transmission. Chromosome 6 was transmitted at 29.6% and 81.5% frequency in populations 2705 and 2701 respectively. The present study showed that molecular markers and molecular cytogenetics applied in this study were most efficient and rapid because a pre-selection for the desired genotypes was possible by screening a population with chromosome-specific markers for the presence of one tomato chromosome at a time.

Keywords Protoplast fusion · Monosomic addition lines · *Solanum tuberosum* · *Lycopsesicon esculentum* · GISH · RFLP

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Introduction

Chromosomes from distantly related species and genera have often been added into a crop plant for breeding as well as for the purpose of fundamental studies. Among many examples, alien chromosome additions have been extensively exploited in crops such as wheat, sugarcane, rice and grasses, among others, where interspecific and intergeneric hybridization can be performed by sexual methods more easily as compared to dicotyledonous taxa (Hadley and Openshaw 1980; Jiang et al. 1994; Multaini et al. 1994). Unlike in monocotyledonous plants, where hybridization using protoplast fusion is difficult, in several dicotyledonous species somatic hybridization has been used for producing intergeneric hybrids (Sigareva et al. 1999; Arumugam et al. 2000; Ren et al. 2000). Among these, somatic hybridization between distant species and genera of the family Solanaceae has received considerable attention (Shepard et al. 1983; Hassanpour-Estahbanati et al. 1986; Derks et al. 1992; Gavrilenko et al. 1992; Chetelat and Meglic 2000). Despite the success achieved in producing numerous interspecific and intergeneric combinations of somatic hybrids within the Solanaceae, there are only a few instances in which the fusion hybrids have been successfully backcrossed to any of the fusion parents. Some examples are, however, the successful backcrossing of the potato (+) tomato fusion hybrids to potato (Jacobsen et al. 1994; Garriga-Calderé et al. 1997), *Solanum tuberosum* (+) *Solanum brividens* (McGrath et al. 1994), *Solanum etuberosum* and *S. tuberosum*×*Solanum berthaultii* (Novy and Helgeson, 1994) and *S. tuberosum* (+) *Solanum bulbocastanum* (Naess et al. 2000). From the analysis of BC_2 and BC_3 progenies it has been demonstrated that the individual alien tomato chromosomes were transmitted through the female parent at variable frequencies (Garriga-Calderé et al. 1998). Based on RFLP and GISH analyses, single tomato chromosome addition lines of potato were identified (Garriga-Calderé et al. 1998). Seven of the possible 12 monosomic alien tomato chromosome addition lines of potato were earlier identified

in BC_2 populations. (Garriga-Calderé et al. 1998). Obviously, this opened the prospect for establishing a complete series of monosomic tomato alien chromosome addition lines of the cultivated potato. Monosomic alien addition lines in different crops have already been created. The main reasons for making alien addition lines is the introgression of genes of interest from distant or wild relatives into the cultivated crops (Chetelat et al. 1998; Khrustaleva and Kik 2000; Friebe et al. 2000) and the construction of chromosome-specific libraries (Schmidt et al. 1990; Ananiev et al. 1997) among others. In order to achieve this aim in potato, an attempt was made to complete the series through the identification of the remaining five different alien tomato chromosome additions. Using a combination of RFLP and GISH analyses in BC_3 populations, a complete series was established and is reported in this article.

Materials and methods

Plant material

The three selected BC_2 plants containing at least one of the five missing alien tomato chromosomes were crossed with two different tetraploid potato pollinators (6704–3 and 6704–13). The berries were harvested on ripening to collect the seeds. These seeds were used for producing BC_3 populations. The BC_2 plants possessed different numbers of alien tomato chromosomes which were as follows: 2103–5 (chrs. 1 and 3), 6731–4 (chrs. 3,6,8 and 9); 2303–5 (chrs. 6,7 and 11). The four BC_3 populations (2808, 2520, 2701 and 2705) consisting of 105 plants, were used for RFLP and GISH analyses. The history of the plant material used for the identification of the five new monosomic alien addition lines is given in Table 1.

RFLP analysis

For RFLP analysis, DNA was isolated from young leaves according to Rogers and Bendich (1988). The procedures for DNA digestion and Southern hybridization were according to Kreike et al. (1990). The DNA was digested with *Eco*RI or *Eco*RV enzymes in order to identify polymorphisms for tomato and potato genomes in the presence of alien tomato chromosome additions. For achieving clear polymorphisms, tomato chromosome-specific probes were used. Two criteria were taken into consideration to establish a polymorphism: (1) a clear-cut difference between potato and tomato polymorphic bands that became evident after hybridization with the probe DNA, and (2) the tomato polymorphic band was completely absent in potato. The tomato chromosome-specific probes used were: TG53, TG366, TG23, TG118, TG438, TG160, TG8 and Ssp29 corresponding to chromosomes 1, 3, 5, 6, 7, 8, 9 and 11 respectively which were present in the BC_2 female parents. Prof. S.D. Tanksley, Cornell University, N.Y., U.S.A., kindly provided the TG probes.

Radioactive RFLP analysis was used and tomato probes were labeled with ^{32}P by random prime labeling according to the procedures described by the manufacturer (Amersham). After Southern blotting, the blot was pre-hybridized with Church buffer (0.36 M Na_2HPO_4, 0.14 M $NaH_2PO_4.H_2O$), 1 mM of EDTA, 7%SDS (dodecylsulfate sodium salt) at 65°C. For hybridization, 10 ml of fresh Church buffer with the labelled probe was added for overnight hybridization at 65°C. The next day, keeping stringency to 0.2× SSC and 0.5% SDS at 65°C, the blot was washed twice. Blots were exposed to the films depending on the activity level after stringency washings which ranged from 2 days to 1 week.

GISH

For analysing chromosome constitution, the root tips were harvested in the morning from in vitro grown plants. The roots tips were treated with 2 mM 8-hydroxyquinoline solution for 2–5 h at 18°C in the dark and fixed in ethanol-acetic acid (3:1). Young anthers with suitable meiotic division stages were fixed in 3:1 ethanol:acetic acid solution during the morning hours. To assess the required stage at meiosis a single anther from a selected bud was squashed and stained in aceto-carmine on a slide and examined under a microscope. The remaining anthers of the selected buds were immediately fixed in the ethanol-acetic acid (3:1) mixture. For storage, the root tips or anthers were transferred to 70% ethanol and stored at −20°C. The protocol followed for digesting both the tissues (roots and anthers) with the pectolytic enzyme mixture was similar to that of Jacobsen et al. (1995). Chromosome preparations were made according to the method described by Zhong et al. (1996).

The procedures for pre-treatments of chromosome preparations, DNA denaturation, in situ hybridization and detection were according of Kuipers et al. (1997). Tomato genomic DNA used as a probe was sonicated to a fragment size of 5–10 kb and labelled with digoxigenin following a standard nick-translation protocol (Boehringer Mannheim). The potato DNA that was used for blocking was autoclaved for 5 min to a fragment size of 100–500 bp. Hybridization was conducted overnight at 37°C.

Digoxigenin-11-dUTP was detected with anti-digoxigenin-fluorescein (20 µg/ml) (Boehringer Mannheim) and amplification was done by rabbit-anti-sheep fluorescein (20 µg/ml) (Vector laboratories). Selected cells were photographed on Fuji 400 ISO colour negative film with an Axiophot microscope equipped with UV light and appropriate filters. Negatives were scanned at 500 dpi and images were optimized with routinely used image-processing software.

Results

Identification of the five new monosomic alien additions in BC_3 populations

In a previous investigation, seven of the possible 12 monosomic alien tomato chromosome addition lines were identified in BC_2 populations. These were for

Table 1 Parentage of the BC_3 potato progenies from which the five new monosomic tomato alien chromosome addition lines were identified

Population	Pollinator	BC_1 plants	Pollinator	BC_2 plants	Pollinator	BC_3 population	Chromosome addition
C31–17–24	× AM66.42	→ 6739	× AM66.42	→ 2103–5	× 6704–3	→ 2808	3
C31–17–1	× AM66.42	→ 6701	× 6020.22	→ 6731–4	× 6704–3	→ 2520	9
C31–17–5	× AM66.42	→ 2002	× 6706–2	→ 2303–5	× 6704–13	→ 2701	7 & 11
					× AM66.42	→ 2705	5

Table 2 Identification of alien tomato chromosomes (3, 5, 7, 9 and 11) in different BC_3 potato populations and the frequencies (%) of their transmission based on RFLPs

[a] = one plant was a disomic addition for chromosome 11 after GISH analysis

Population	No. of plants analysed	No. of potato plants monosomic to the alien tomato chromosomes				
		3	5	7	9	11
2808	31	1 (3.2)				
2705	27		1 (3.7)			
2701	38			1 (2.6)		2 (5.2)[a]
2520	9				3 (33.3)	

tomato chromosomes 1, 2, 4, 6, 8, 10 and 12. In order to complete the series, attention was focused only on the identification of the remaining five chromosomes 3, 5, 7, 9 and 11 in BC_3 populations. Previous experience had shown an enormous difference in the frequency of transmission of different tomato chromosomes in different populations. Moreover, there were also considerable differences between the three BC_2 populations that were investigated for the isolation of the earlier selected seven monosomics. In view of this, a careful choice of the four BC_3 populations (Table 1, see below), that had the potential of possessing the missing monosomic additions, was made.

RFLP analysis

This choice of the BC_2 parents for backcrossing to obtain BC_3 populations was based on the alien chromosome composition determined through RFLP and GISH analyses. Using 105 BC_3 plants, representing four different populations, all the remaining five classes of alien tomato monosomic addition lines were identified among seven candidate BC_3 plants (Table 2). The frequency of monosomics varied between 3.2 and 33.3%. It should be pointed out that one BC3 population (2701) provided putative monosomics for the desired tomato chromosomes 7 and 11. This obviously completed the monosomic alien tomato chromosome-addition series based on RFLP analysis (Fig. 1). However, RFLP analysis alone was not adequate for discriminating between real monosomic additions and potential disomic additions. Therefore, an additional cytological analysis of somatic chromosomes was conducted using GISH to re-confirm the presence of a single alien tomato chromosome.

GISH analysis

Out of the eight potential monosomic additions that were detected, all but one turned out to be single chromosome additions (Fig. 2A). The exceptional one (2701–14), although identified as a monosomic through RFLP analysis, turned out to be a disomic addition for alien tomato chromosome 11 based on GISH (Fig. 2B). Unlike the monosomic additions, the disomic addition line was expected to be more useful for the high frequency of transmission of the alien chromosome in the sexual progeny. This was due to the fact that the presence of a pair of

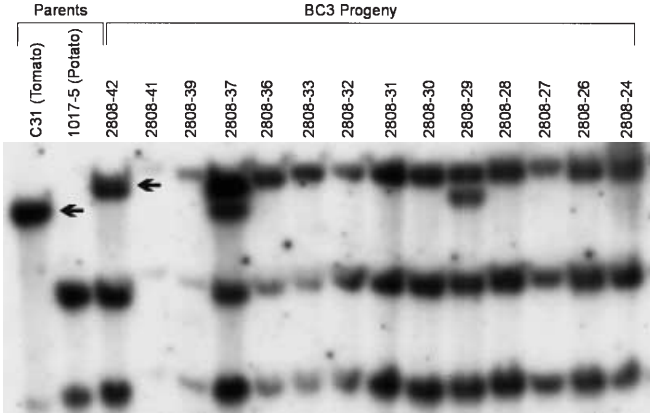

Fig. 1 A representative autoradiogram of a Southern blot after *Eco*RI digestion, probed with TG366, specific for tomato chromosome 3. The polymorphism between tomato (C31) and potato (1017–5) is clearly visible (*arrow*). Both bands are present in the BC_3 plants 2808–37 and 2808–29. Hence these plants carry tomato chromosome 3

homologous alien tomato chromosomes in potato might facilitate their normal segregation during meiosis. In view of this, the meiotic behaviour of the disomic chromosome addition genotype 2701–14 was investigated through GISH (Fig. 2C, D). A notable feature was that this chromosome 11 pair of tomato formed consistently two univalents at the metaphase-I stage (Fig. 2E) instead of a bivalent. In order to monitor the pairing behaviour of this disomic addition, chromosomes at the pachytene stage were analysed. Although pairing was observed in about half of the pollen mother cells, in other cases the homologous chromosomes were either unpaired or only partially connected. In 33 cells that were analysed 18 showed clear pairing, whereas in the other 15 either there was no pairing or the chromosomes were partially paired (Fig. 2C, D). At late prophase-I and metaphase-I stages only univalents of alien tomato chromosomes were observed. Obviously, the paired tomato chromosomes showed a precocious separation of the bivalent as compared to the potato chromosomes. This might result from the asynchrony of chromosome behaviour of the alien genomes in general. For example, the centromere divisions and chromosome movements of the alien genomes were at odds also during later stages of meiosis (Fig. 2E–H). Because of the formation of univalents the movement of the two univalents of tomato chromosomes was haphazard during anaphase-I as well as of anaphase-II (Table 3). The ultimate result was that the anticipated

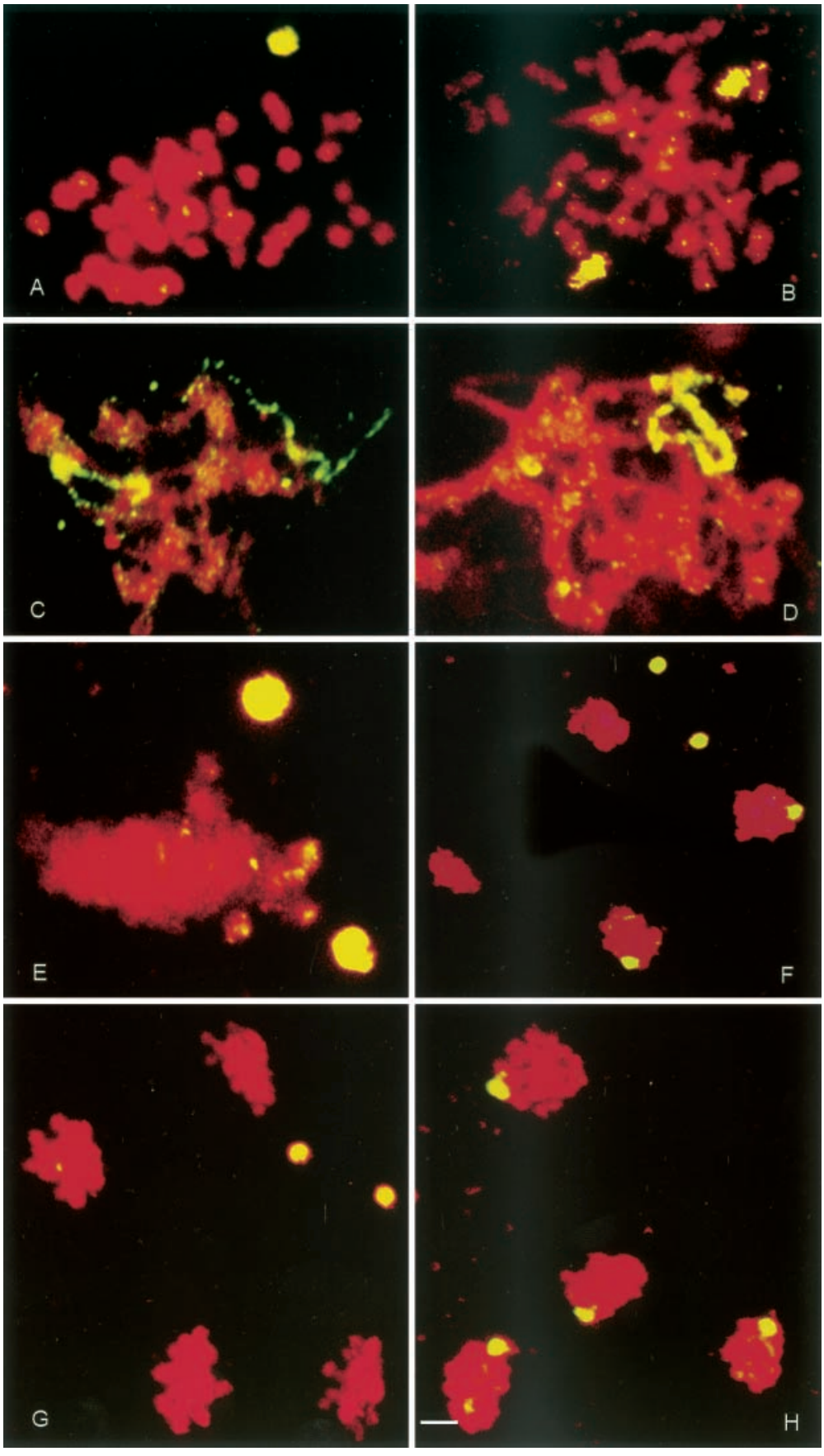

Fig. 2 GISH on mitotic and meiotic cells. **A–B** Representatives of a typical monosomic and disomic addition lines. **C–D** Pachytene stages of a disomic (2701–14) for chromosome 11 showing two tomato chromosomes (yellowish green) not pairing at all and the same two chromosomes (yellow) pairing normally, the red ones are potato chromosomes. **E** Metaphase I stage for disomic (2701–14), the two chromosomes (yellow) fall apart and are not oriented on the equatorial plate with potato chromosomes. **F** Telophase II stage of disomic (2701–14) showing two tomato chromosomes (yellow) as laggards whereas other two chromosomes have moved to two poles. **G** Disomic (2701–14), the tomato chromosomes (yellow) are still undivided indicating asynchrony of centromere division. **H** In disomic (2701–14) at telophase II stage the chromosomes divide equally. In all cases the potato chromosomes are stained orange red because of the counter-staining of propidium iodide. The bar represents 10 μm approximately

Table 3 Distribution (%) of alien tomato chromosomes in the potato line 2701–14 disomic for tomato chromosome 11

Meiotic stage	No. of cells analysed	Alien chromosome distribution to poles				
		1–1	2–0	One lagging	Both lagging	
Anaphase-I/ telophase-I	56	10 (18.0)	15 (27.0)	24 (43.0)	7 (12.5)	
		1 each to 4 poles	1 each to 2 poles +2 lagging	2 each to 2 poles	1 each to 2 poles +2 in 1 pole	1 each to 3 poles +1 lagging
Anaphase-II/ telophase-II	68	16 (23.5)	19 (28.0)	19 (28.0)	6 (9.0)	9 (13.0)

Table 4 Summary of transmission frequencies (%) of tomato chromosomes from BC_2 plants to BC_3 populations

Population	No. of plants	Mode of transmission of chromosomes								
		Together		Individual			Overall			
2802	31	**1 & 3** 3 (9.6)		**1** 13 (41.9)	**3** 1 (3.2)		**1** 16 (51.6)	**3** 4 (12.9)		
2705	27	**5 & 6** 2 (7.4)		**5** 1 (3.7)	**6** 6 (22.2)		**5** 3 (11.1)	**6** 8 (29.6)		
2701	38	**6 & 7** 3 (7.8)	**6 & 11** 2 (5.2)	**6** 27 (71.0)	**7** 1 (2.6)	**11** 1 (2.6)	**6** 31 (81.5)	**7** 4 (10.5)	**11** 3 (7.8)	
2520	9	**8 & 9** 1 (11.1)		**8** 1 (11.1)	**9** 3 (33.3)		**8** 2 (22.2)	**9** 4 (44.4)		

(regular) meiotic behaviour of the disomic addition was lacking in this plant. Additional RFLP studies on 2701–14 did confirm the earlier observation of the presence of only alien tomato chromosome 11 in this plant (data not shown).

Frequency of transmission of alien tomato chromosomes from BC_2 plants to BC_3 populations

The frequencies of occurrence of the five alien monosomic additions were clearly variable and ranged between 2.6% (chromosome 7) and 33.3% (chromosome 9) in the different BC_3 populations (Table 2). Besides the five tomato chromosomes (3, 5, 7, 9, 11) that were identified as monosomics, other tomato chromosomes (1, 6 and 8) were also segregating in the same BC_3 populations. The frequency of transmission of these chromosomes from BC_2 plants to BC_3 populations was also calculated (Table 4). Each individual chromosome was transmitted with a different frequency in all combinations. There were two tomato chromosomes in three populations and three in one population that were transmitted with variable frequencies. The transmission frequencies of chromosomes 1,6 and 8 were relatively high. This might have decreased or affected the transmission of the desired chromosomes 3, 5, 7, 9 and 11 as single copies in individual offspring plants. However, all desired chromosomes were transmitted from BC_2 plants to BC_3 populations. All combinations of alien chromosomes were observed in four BC_3 offsprings except in population 2701, where the combination of chromosomes (6+7) and (6+7+11) was missing (Table 4).

In order to complete the whole series of alien tomato chromosome-addition lines of potato, with different genotypes of the fusion hybrids, the BC_1 and BC_2 plants had to be used as female parents with different potato pollinators (Table 5). Thus, the genetic background of this alien addition series is heterogeneous. However, the identification was completed by screening BC_2 populations for seven different monosomic additions, and BC_3 populations for the additional five new monosomics. In this process, however, not just one genotype of a monosomic addition but several genotypes for most of the chromosomes, and for two chromosomes (10 and 11) disomic additions also became available (Table 5).

Characteristics of monosomic additions like morphology and fertility were also monitored. The potato addition lines for all 12 tomato chromosomes resembled male potato parents in leaf shape, colour and morphology. The flower shape and colour was also similar to potato plants. A drawback of these addition lines is that due to the tetraploid background of the potato, the alien tomato chromosomes did not express any tomato-specific phenotypes. The growth and fertility was not measured but, in general, all BC_3 plants showed vigorous growth and were male- and-female fertile. The homoeologous pairing between potato and tomato chromosomes had already been monitored in previous studies where the first seven monosomic addition lines were selected and, therefore, this aspect was not further investigated here.

Table 5 An overview of the establishment of a complete series of monosomic alien tomato chromosome additions from BC_2 and BC_3 generations to the cultivated potato

Fusion Hybrid	Pollinator	BC_1	Pollinator	BC_2	Pollinator	BC_3	Tomato chromosomes	Number of monosomic additions found	Chr. transmission ![b] BC_1 to BC_2 in (%)	Remarks
C31–17–24	AM66.42	6739	AM66.42	TMA[a]	×	×	1	2103–1 2103–2 2103–4	8.0–41.4	Monosomics in (BC_2)
C31–17–5	6704–1	2003	6707–7	TMA	×	×	2	2102–5 2102–9 2403–6	6.9–92.0	Monosomics in (BC_2)
C31–17–24	AM66.42	6739	AM66.42	2103–5	6704–3	TMA	3	2808–37	3.4–28.0	Monosomics in (BC_3)
C31–17–24	AM66.42	6739	AM66.42	TMA	×	×	4	2101–7 2101–8 2103–10	6.9–28.0	Monosomic in (BC_2)
C31–17–5	AM66.42	2002	6706–2	2303–5	AM66.42	TMA	5	2705–4	10.3–11.6	Monosomic in (BC_3)
C31–17–5	AM66.42	2002	Desiree	TMA	×	×	6	2301–5 2301–6 2301–14 2301–18 2302–1 2303–3	13.8–88.4	Monosomics in (BC_2)
C31–17–5	AM66.42	2002	6706–2	2303–5	6704–13	TMA	7	2701–9	4.0–13.9	Monosomic in (BC_3)
C31–17–5	AM66.42	2002	Desiree	TMA	×	×	8	2301–2 2301–27	20.9–36.0	Monosomics in (BC_2)
C31–17–1	AM66.42	6701	6020.22	6731–4	6704–3	TMA	9	2520–1 2520–4 2521–2	0.0–32.0	Monosomics in (BC_3)
C31–17–24	AM66.42	6739	6706–1	TMA	×	×	10	2101–1	10.3–20.0	Disomic in (BC_2)
C31–17–5	AM66.42	2002	6706–2	2303–5	6704–13	TMA	11	2701–6 2701–14	8.0–13.8	Monosomic in (BC_3) Disomic in (BC_3)
C31–17–24	AM66.42	6739	6704–6	TMA	×	×	12	2102–6 2303–9	13.8–18.6	Monosomics in (BC_2)

[a] TMA=tomato monosomic addition was found [b] !=data from Garriga-Calderé et al. (1998)

Discussion

This investigation demonstrates that, through a combination of RFLP and GISH analyses, the entire series of alien tomato chromosome addition lines in potato could be completed relatively easily. The combined effectiveness of these two techniques was already proven by Jacobsen et al. (1995) and Garriga-Calderé et al. (1998) for the establishment of tomato monosomic addition lines. If this were to be established through conventional cytogenetic techniques it would have been extremely laborious and frustrating. Laborious because the chromosomes of tomato are too small for chromosome identification in somatic cells and pachytene chromosomes are difficult to prepare for cytological analysis (Ramanna and Prakken 1967). It would have been frustrating because numerous genotypes would have been identified for chromosomes with higher frequencies of transmission. In other words, a pre-selection of a potentially useful genotype in the BC_1 and BC_2 generation would not be easy with the conventional approach. The advent of RFLP analysis has changed this situation drastically because the use of chromosome-specific RFLP markers enabled us to make a pre-selection. During this process, it was possible to establish whether only one or more alien chromosomes were present in a given genotype. When only one alien chromosome was present, it was possible to identify such genotypes in more detail using more-appropriate probes as well as through GISH. The combination of these two molecular techniques has been used

earlier and proved successful both in sexual and somatic hybrids between *Lycopersicon esculentum* and *Solaum lycopersicoides* (Escalante et al. 1998) as well as in hybrids of *Gibasis* (Parokonny et al. 1992).

Although a combination of molecular techniques was crucial in establishing a complete series of monosomic alien addition lines, an appropriate strategy of using proper parents and populations was equally important. This is because of the following two facts: (1) some of the alien chromosomes are transmitted through the female parent at a very low frequency or not at all (see Garriga-Calderé et al. 1998; Haider Ali et al., submitted); (2) conversely, some of the chromosomes are transmitted at an extremely high frequency, so much so that, in some cases, 100% of the progeny consisted of such an alien chromosome as in the case of chromosome 6 of tomato in a particular population (Haider Ali et al., submitted). The differences in the transmission rate of individual alien chromosomes in monosomic alien addition lines have been reported earlier by researchers in other crops. Such examples include rice (Jena and Khush 1989), cotton (Rooney et al. 1991), tobacco (Suen et al. 1997) and tomato (Chetelat et al. 1998). The characteristic rate of transmission of individual chromosomes was also evident to some extent in this study. The chromosomes were transmitted at different frequencies in different combinations and this shed light on the peculiar genetic behaviour of a chromosome. For example, chromosome 1 remains superior in transmission to chromosome 3 and together they have a very low frequency of transmission which seems as if the gametes that carry these two chromosomes together are less viable. Another example is chromosome 6, which in population 2705 had 29.6% transmission, and in population 2701 was transmitted at a much higher rate, 81.5% (Table 4). In an earlier study chromosome 8 could not be recovered at all in a BC_3 progeny (Haider Ali et al., submitted), whereas in this case there is a moderate transmission rate for chromosome 8 (22.2%). There is no clear explanation for the variable rate of transmission frequencies of tomato chromosomes in a potato background. The only valid explanation, and this also seems convincing, is that the different genetic background influences the transmission of alien chromosomes, and each tomato chromosome has a unique genetic makeup that makes it exclusive in its behaviour. The GISH analysis of the disomic addition (Fig. 2C–H) at the pachytene and metaphase-I stages also showed the irregular pairing behaviour of homologous chromosomes and, in later stages, their meiotic behaviour was highly disturbed.

Both of these aspects, viz., the low transmission for certain chromosomes (3.2% for chromosome 3) as well as the high transmission rate for other chromosomes (33.3% for chromosome 9; Table 2), can complicate the selection and identification of the desired alien addition line. However, the fertility of all alien addition lines was reasonably high and the alien chromosomes were transmitted to the next generation in most cases. Nevertheless, in view of the enormous amount of variation in the rate of transmission of individual chromosomes, the selection of proper backcross populations was important.

Previous cytological investigation on other monosomic alien addition lines has shown that there was very little or no homoeologous recombination between potato and tomato chromosomes (Garriga-Calderé et al. 1998). Therefore, no attempt was made during this investigation to establish whether there were any recombinant chromosomes among the newly selected alien tomato chromosome addition lines. It should be noted, however, that it is extremely difficult, if not impossible, to detect cytogenetically the recombinant chromosomes involving a potato and tomato chromosome. This is because of the very small lengths of the euchromatic segments that are present in the somatic chromosomes of both species. Apart from the smallness of the euchromatic segments, there might be another theoretical reason why homoeologous recombinants cannot be detected in potato and tomato. It has been well-established that the distribution of repetitive DNA sequences on chromosomes is a crucial factor for differentiating genomes and chromosomes through GISH (Parokonny et al. 1992; D'Hont et al. 2000). Since there is very little or no repetitive DNA present in euchromatic regions, GISH cannot easily resolve recombinant segments in the species involved in the present material. There is no solid justification for the low homoeologous pairing between potato and tomato chromosomes and, subsequently, the low transmission rate of alien chromosomes. However, a convincing answer is that the presence of two genomes of tomato and four genomes of potato (for details see Garriga-Calderé et al. 1997) was not the best situation to stimulate homoeologous pairing of potato and tomato chromosomes. This has been observed frequently in interspecific hybrids and their backcross progenies of *Alstroemeria* (Kamstra et al. 1999) and *Lilium* (Lim et al. 2000) where the presence of only one genome of both species influenced homoeologous pairing positively.

The completion of monosomic tomato chromosome addition lines of potato adds one more instance to the list of only a few crops in which such a series has been identified (see for reviews Khush 1973; Garriga-Calderé et al. 1998). Such a series has been shown to be highly useful in assigning desirable genes to respective chromosomes and in the breeding of rice (Brar and Khush 1997). Apart from the use of alien addition lines in breeding (Janssen et al. 1997) they can also be useful for the study of chromosome organization (Kamstra et al. 1997; Zhong et al. 1998). The monosomic or disomic alien tomato chromosome addition lines are also helpful in further cytological and molecular studies. Classification of respective polymorphic and non-polymorphic RFLP and AFLP markers on a particular chromosome will provide the possibility to know whether a chromosome is complete or not. Localization of BAC's and YACs on monosomic alien chromosomes will help facilitate the characterization and isolation of genes of interest.

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